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The effect of different polychlorinated biphenyls on two aquatic models, the green alga *Pseudokirchneriella subcapitata* and the haemocytes from the European abalone *Haliotis tuberculata*

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HIGHLIGHTS

- Comparison of ecotoxicity of PCBs on a green algae and the haemocytes from gastropod.
- Green algae median EC_{50} values ranged from 0.34 μM to more than 100 μM.
- Abalone EC_{50} values ranged from 1.67 μM for PCB153 to 89 μM for PCB28.
- No differences between the DL and NDL PCBs ecotoxicities regardless of the model used.
- Our results demonstrated that the ecotoxicities of PCBs were model dependent.

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ABSTRACT

The present study was conducted to determine the toxicity of different polychlorinated biphenyls (PCBs) on the green algae, *Pseudokirchneriella subcapitata* and the haemocytes from the European abalone, *Haliotis tuberculata*. Using the algal growth inhibition test, the green algae median Effective Concentration (EC_{50}) values ranged from 0.34 μM for PCB28 to more than 100 μM for PCBs 101 and 153. Considering the MTT viability test, the abalone EC_{50} values ranged from 1.67 μM for PCB153 to 89 μM for PCB28. Our results in contrast to previous observation in vertebrates did not show significant differences between the dioxin like- and non dioxin like-PCBs toxicities regardless of the model used. However, our results demonstrated that the toxicities of PCBs were species dependent. For example, PCB28 was the most toxic compound for *P. subcapitata* whereas PCBs 1, 180 and 153 were less toxic for that species. On the contrary, PCB153 was reported as the most toxic for *H. tuberculata* haemocytes and PCB28 the least toxic. To investigate the mode of action of these compounds, we used an *in silico* method. Our results suggested that PCBs have a non-specific mode of action (e.g., narcosis) on green algae, and another mode of action, probably more specific than narcosis, was reported for PCBs on the abalone haemocytes.

1. Introduction

For the past several decades, human activities have discharged large quantities of environmental pollutants. Some of the pollutants introduced into terrestrial and aquatic ecosystems may possess toxic effects. Some of them belong to persistent organic pollutants (POPs) which are organic compounds that are resistant to biodegradation. As a consequence, these molecules persist in the environment, bioaccumulate in the biota and in some case are magnified through the food chain (Connolly, 1991; Crinnion, 2011a).

Among POPs, polychlorobiphenyls (PCBs) are compounds of anthropogenic origin that possess 209 congeners differing in the number and position of chlorine atoms. Due to their physical

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chemicals properties, PCBs were used as insulators in transformers, hydraulic fluids, paint additives, fire retardants, and pesticide extenders (Eisler and Belisle, 1996; Erickson and Kaley, 2011). Although their production ended in the late 1980s, they are still present in the environment and, thus, can give rise to ecological risks resulting in a close monitoring of these contaminants worldwide (Jensen et al., 1969; Howell et al., 2008; Carvalho et al., 2009; Hauck et al., 2010; Montory et al., 2011). Although the concentrations of these compounds in the environment have been declining, they are still listed as Priority Substances within the EU Water Framework Directive (Directive 2013/39/EU, 2013). These pollutants are reported to cause detrimental effects to wildlife and humans, including carcinogenesis, mutagenesis, endocrine disruption, and immunotoxicity (Safe, 1984; Eisler and Belisle, 1996; ATSDR, 2000; Crinnion, 2011b).

Due to their (eco)toxicological effects and their magnification along food chains, they still pose a serious environmental concern (Sinkkonen and Paasivirta, 2000; Beyer and Biziuk, 2009). For example, many studies have shown the accumulation ability of PCBs by phytoplankton, which are implicated in food web alteration and resultant damages to commercially important fisheries (O'Connors et al., 1978).

In Europe, seven PCBs were chosen by the European Commission as being of PCB research and study primarily because of their abundance in the environment and their toxicological properties (McFarland and Clarke, 1989). The impacts of PCBs on the environment and biota are due to the individual components of this mixture and their additive and/or nonadditive interactions among themselves and other chemical classes of pollutants (Giesy and Kannan, 1998). It has been shown that congener profiles of PCBs in environmental media can be changed significantly from the profiles of commercial mixtures (e.g. Aroclor). Therefore, risk assessment based on commercial mixture may not be representative of the risks posed by PCBs (WHO, 2001). Several studies have demonstrated differences in both mechanisms and toxic potentials of individual PCB congeners (Henry and DeVito, 2003). Thus, the hazard assessments of PCBs require ecotoxicological studies on individual PCB congeners (WHO, 2001).

The interactions of PCBs with the biota or properties of individual congeners like bioaccumulation, binding to biological receptors (Boon et al., 1997), toxicity (Eriksson et al., 2002) or metabolism (Yunker et al., 2011) depend upon both the number of chlorines and their position around the biphenyls rings. For many years, concerns of PCB toxicity have focused on dioxin-like actions (immunosuppression and carcinogenesis) mediated via activation of the aryl hydrocarbon receptor and the induction of cytochrome P450 (Safe, 1999, for revue). These effects are primarily due to congeners that have chlorines only in the *meta* and *para* positions which can assume a coplanar dioxin-like configuration. Congeners with chlorines in the *ortho* position are energetically dissuaded from assuming a coplanar configuration. Because of this diversity in biological activities, PCBs show a wide range of potential effects (Fisher et al., 1998; ATSDR, 2000; Henry and DeVito, 2003). Although the mechanisms of action of PCBs are relatively well known in vertebrates, the biological effects and the mechanisms of action of PCBs are not yet completely understood in invertebrate organisms or phytoplankton (WHO, 2001).

The purpose of this study is to generate new ecotoxicologic data of individual PCBs based on their structural properties on phytoplankton and invertebrate and to propose a possible interpretation in terms of mode of action. Phytoplankton forms the basis of many aquatic food chains. Most studies on the impact of PCBs on phytoplankton focuses on bioaccumulation capacities and the knowledge concerning their toxicity is in state confusing (e.g. photosynthetic mechanism, chloroplast alteration) (Conner and Mahanty, 1979). In the present study, we have chosen to use the green algae *Pseudokirchneriella subcapitata* (formerly named *Selenastrum capricornutum*) as a biological model. This alga is commonly used as a model for standard toxicity tests (OECD, 2006; US-EPA, 2002; ISO 8692, 2004). Its use is widespread in ecotoxicological tests mainly because of its ease of cultivation, rapid growth (Nygaard et al., 1986) and sensitivity to a wide range of contaminants (Kamaya et al., 2003; Colombo et al., 2008; Aruoja et al., 2009; Pretti et al., 2009; Wik et al., 2009). *P. subcapitata* is an excellent indicator organism of pollution in aquatic ecosystems where trace contaminants are difficult to analyse directly. However, to our knowledge, few studies exist in the literature on the effect of PCBs on these green algae. For example, results obtained by Mayer et al. (1998) indicated the effective concentration (EC_{50}) of three PCBs (PCB31, 48 and 105) after 48 h or 72 h of exposure. These authors calculated EC_{50} values of 241 nM and 14 nM for PCB31 and 105, respectively, after 24 h exposure. For PCB48, an EC_{50} value of 134 nM was obtained after an exposure of 72 h.

Additionally, we used a non-conventional model to compare the effects of PCBs: the haemocytes from the European abalone, *Haliotis tuberculata*. Haemocytes are cells that are continually exposed to the external environment due to the open circulatory system of molluscs. To assess the effects of PCBs on the abalone immune response, an *in vitro* approach was chosen. *In vitro* approaches are alternative experimental methods to whole- animal testing that are employed because of the reduced use of animals, capability for standardisation, low cost and rapid performance associated with these methods, in addition to ethical considerations (Schirmer, 2006; Shuilleabhain et al., 2006). Although *in vitro* assays may not always reflect the true *in vivo* situation, which is more complex, they undoubtedly provide important data to determine mechanisms at the molecular and the cellular levels (Binelli et al., 2009). Indeed, cell cultures have allowed cells to be studied in a controlled environment and in isolation from the multiple physiological systems that regulate their activities *in vivo*. It is for this reason that marine invertebrate cell cultures had been developed during the last decade to study physiological processes (Brousseau et al., 2000; Olabarrieta et al., 2001; Canesi et al., 2003; Gagnaire et al., 2006; Duchemin et al., 2008; Mottin et al., 2010; Latire et al., 2012). However, few investigations have reported the effects of PCBs on marine molluscs at the cellular level. For example, Gagnaire et al. (2006) have investigated the *in vitro* effects of PCB77 and 153 on the haemocytes from the oyster *Crassostrea gigas*. These authors demonstrated that PCB 77 induced a significant decrease of lysosome-positive haemocyte percentage after an incubation periods of 4 h at 6 and 60 μM., but no effects were recorded on other tested parameters (e.g. cell viability, phagocytose activity, ROS production). However PCB153 had no effects suggesting that the effects of PCBs to be congener-specific in mollusc. In mussel haemocytes (Canesi et al., 2003), PCB47 and 153 can alter immune parameters (e.g. microbial activity and lysosomal enzyme release, respectively). Additionally, both PCBs and PCB77 reduced hemocyte lysosomal membrane stability. Moreover these PCBs act on molecular targets involved in signal transduction (e.g. tyrosine kinase-mediated cell signalling) corresponding to those found in human neutrophils (Canesi et al., 2003). These results suggest that haemocytes represent a useful model for evaluating the potential effect of PCB.

In order to assess the toxic properties of different congeners, we chose representative PCBs among the 209 congeners according the number of chlorines and their position around the biphenyls rings and their potential toxicity as a function of environmental frequency (Rein and Bittens, 2004). In their study, Rein and Bittens defined 4 groups of PCBs: "highest toxic potential" (group A), "high to moderate toxic potential" (group B), "low toxic potential" (group C) and "no toxic potential reported" (group D). In our study, we analyse the effects of PCBs belonging to each group (PCB77,

118, 138, 169 (group A); 101, 153, 180 (group B); 15, 52, 80, 136 (group C); 8, 28 (group D)) on *P. subcapitata* and haemocyte from *H. tuberculata*. Moreover, the effects of 3 PCBs not reported in these groups (PCBs 1, 3, 107) were investigated.

2. Materials and methods

2.1. Chemicals

PCB1, 3, 8, 15, 28, 52, 77, 101, 138 and 153 (Table 1) were provided by Sigma–Aldrich (France) from SUPELCO Analytical (USA) as 10 mM solutions in DMSO (the purity of all compounds was 99.9%). PCB80, 107, 118, 136, 169, and 180 (Table 1) were purchased as powders (purity > 98%) from LGC Standards (Molsheim, France) All compounds were dissolved in DMSO (purity > 99.5%).

2.2. Pseudokirchneriella subcapitata growth inhibition tests

The growth inhibition test with the unicellular algae *P. subcapitata* was adapted from the ISO standard (NF T90-375, 1998). Batch cultures were incubated with tested compounds, i.e., 12 NDL-PCBs (PCB1, 3, 8, 15, 28, 52, 80, 101, 136, 138, 153, and 180) or 2 DL-PCBs (PCB77 and 118) in 96-wells microplates for 72 h. The inhibition of growth was determined at 23 ± 2 °C under continuous illumination of 60 μE m^{-2} s^{-1}. The initial concentration of the inoculum was 2×10^5 cells mL^{-1}. After 72 h, cell density was measured fluorometrically at 485 nm (excitation) and 640 nm (emission). Six PCB concentrations were tested per plate and six replicates per concentration. In each experiment, a control without DMSO and a DMSO control were performed.

2.3. Abalone specimens

Adult *Haliotis tuberculata* abalone (9–11 cm in shell length) were collected by Ormasub® from natural populations on the northern Cotentin peninsula (France). The animals were maintained in natural and continuously aerated seawater at 17 °C and regularly fed with a mixed algal diet (*Laminaria* sp. and *Palmaria* sp.) at the Centre de Recherche en Environnement Côtier (C.R.E.C, Luc-sur-Mer, Basse-Normandie, France). The abalones were acclimated for at least two weeks before the experiments began.

Table 1
Overview of the congeners tested in this study. Log*Kow* values were reported from Yeh and Hong (2002) and Sangster (1994).

Congener	Full name	Log*Kow*
NDL-PCB		
PCB1	2-Chlorobiphenyl	4.38
PCB3	4-Chlorobiphenyl	4.49
PCB8	2,4′-Dichlorobiphenyl	5.14
PCB15	4,4′-Dichlorobiphenyl	5.33
PCB28	2,4,4′-Trichlorobiphenyl	5.68
PCB52	2,2′,5,5′-Tetrachlorobiphenyl	5.81
PCB80	3,3′,5,5′-Tetrachlorobiphenyl	6.85
PCB101	2,2′,4,5,5′-Pentachlorobiphenyl	6.5
PCB107	2,3,3′,4′,5-Pentachlorobiphenyl	6.45
PCB136	2,2′,3,3′,6,6′-Hexachlorobiphenyl	6.81
PCB138	2,2′,3,4,4′,5′-Hexachlorobiphenyl	6.32
PCB153	2,2′,4,4′,5,5′-Hexachlorobiphenyl	6.9
PCB180	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	6.56
DL-PCB		
PCB77	3,3′,4,4′-Tetrachlorobiphenyl	6.35
PCB118	2,3′,4,4′,5-Pentachlorobiphenyl	6.49
PCB169	3,3′,4,4′,5,5′-Hexachlorobiphenyl	7.01

2.4. Abalone haemocyte primary cell cultures

Haemocytes were cultured as previously described (Mottin et al., 2010; Latire et al., 2012). Briefly, after making a medio-lateral incision in the abalone foot, haemolymph was collected (10–15 mL per animal) with a 20 mL syringe fitted with a 25-gauge hypodermic needle. Haemolymph was transferred to a sterile tube and diluted 1:4 in cooled sterile anticoagulant modified Alsever's solution (115 mM glucose; 27 mM sodium citrate; 11.5 mM EDTA; and 382 mM NaCl) (Bachère et al., 1988). Haemocytes were rapidly plated at a density of 1.5×10^6 cells per well in 6-well plates into which three volumes of sterile artificial seawater were added. The cultures were maintained at 17 °C in a CO_2-free incubator. After 90 min of incubation, the cells were covered with Hank's sterile 199 medium modified by the addition of 250 mM NaCl, 10 mM KCl, 25 mM $MgSO_4$, 2.5 mM $CaCl_2$ and 10 mM Hepes (final pH of 7.4). The medium was supplemented with 2 mM L-glutamine, 100 μg mL^{-1} streptomycin, 60 μg mL^{-1} penicillin G and 2 mM concanavalin A. The cells were then maintained at 17 °C for 24 h before beginning the experiments.

The medium was replaced, and the cells were then exposed to PCBs. For each concentration, the treatment was performed in quadruplets. For haemocytes, 5 NDL-PCBs (PCB3, 28, 107, 136, and 153) and 2 DL-PCBs (PCB77, and 169) were tested. The medium was changed every day, and the cultures were maintained for ten days. Each experiment was repeated at least three times. In each experiment, a control without DMSO and a DMSO control were performed.

2.5. Haemocyte viability test

Haemocyte viability was estimated using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assay, which is a sensitive and quantitative colorimetric assay that measures the capacity of mitochondrial succinyl dehydrogenase in living cells to convert a yellow substrate (MTT) into a dark blue formazan product (Mosmann, 1983). This test was adapted to molluscan cell cultures by Domart-Coulon et al. (1994). Briefly, 10% (v/v) of the MTT stock solution (5 mg MTT mL^{-1} in PBS) was added to the culture dishes. After 24 h of incubation, an equal volume of isopropanol containing 0.04 N HCl was added to each culture to dissolve the converted formazan. The absorbance was then measured at a wavelength of 570 nm with a 630 nm reference.

2.6. Haemocyte morphological analysis

The potential effects of PCBs on cell morphology were performed as previously described (Mottin et al., 2010; Latire et al., 2012). Haemocytes were cultured as described above on coverslips. Cells were fixed in methanol for 10 min at room temperature and stained with haematoxylin and light green dye. Haemocytes were then incubated for 10 min in acetone and the coverslips were mounted in Roti-Histol® (Roth, Karlsruhe, Germany). Observations were carried out using a Nikon Eclipse 80i light microscope.

2.7. Data analysis

Results were expressed as the means ± S.D. Each experiment was repeated at least three times, and the means were calculated from triplicates of each experiment. The significance of the differences between the mean values was estimated using Kruskall-Wallis and Mann–Whitney tests. Non-linear regressions (using the Hill equation) on the obtained data allowed us to calculate effective concentration for an effect on 50% of the populations tested (EC_{50} values) for each PCB. These regressions were conducted using the Excel® macro REGTOX (Vindimian, 2012). For *in silico* analysis the ecotoxicity-log*Kow* relationship curve was used:

all the regressions were performed using Microsoft Excel and the correlation were studied by Spearman rank tests. The statistical threshold was set at $p < 0.05$.

3. Results

3.1. Effect of PCBs on the growth inhibition of P. subcapitata

Fig. 1A shows the results obtained from a typical experiment (i.e., PCB28). Compared to the control, no significant effects of PCB28 were recorded for the DMSO control or the first concentration tested (0.125 μM). At the PCB28 concentration of 0.25 μM, a mild (22%) but significant ($p < 0.01$) growth inhibition was observed. When *P. subcapitata* were exposed to 0.5 μM PCB28 and higher, a drastic growth inhibition (more than 79% compared to the 100% control) was recorded. Compared to the control, the highest PCB28 concentration (4 μM) decreased algal growth by 99.96% (0.04 ± 0.02% in treatment *vs* 100% for the control). With these data values, a non-linear regression allowed us to calculate an EC_{50} value of 0.34 μM (confidence interval (95%): 0.30–0.38 μM) for PCB28 (Fig. 1A, Table 2A). Table 2 shows the EC_{50} values obtained for each PCB tested.

3.2. Effect of PCBs on the haemocyte viability in Haliotis tuberculata

Fig. 1B shows the results obtained from a typical experiment (i.e., PCB153). Compared to the control, no significant effects of PCB153 were recorded with the DMSO control. A significant ($p < 0.01$) effect was recorded at the first concentration of PCB153 tested (1.25 μM): at this concentration, the viability decreased by 27 % (73 ± 8% in treatment *vs* 100% for the control). When *H. tuberculata* haemocytes were exposed to 2.5 μM and higher of PCB153, a drastic growth inhibition was recorded. Compared to the control, the highest PCB153 concentration (4 μM) decreased algal growth by 97.9 % (2 ± 1% in treatment *vs* 100% for the control). With these data values, a non-linear regression allowed us to calculate the EC_{50} for PCB153. For *H. tuberculata*, the PCB153 EC_{50} was 1.67 μM (confidence interval (95%): 1.48–1.89 μM) (Fig. 1B, Table 2B). Table 2 shows the EC_{50} values obtained for all PCBs tested.

After 10 d of exposure, haemocytes morphology was noticeably affected by the presence of PCBs indicated by their structure (Fig. 2). In the absence of PCBs (control, Fig. 2A), cells were interconnected and presented an elongated shape with large pseudopods. The addition of DMSO to the culture medium (Fig. 2B) did not modify the morphology of the haemocytes. In the presence of PCB28, the haemocyte morphology was also not impacted: the culture wells always contained numerous spreading cells with pseudopods (Fig. 2C). However, in the presence of PCB153, the haemocytes became isolated and morphological modifications could be observed (Fig. 2D).

3.3. Comparison of the effect of PCBs in the different models used

Table 2 shows the EC_{50} values obtained from the two models used in this study. The results from the *P. subcapitata* study showed EC_{50} values higher than 100 μM for 4 PCBs (PCB1, 80, 136, 153) which are all NDL-PCBs. Among the tested toxicants, the most toxic molecule was NDL-PCB28 with an EC_{50} value lower than 1 μM (0.34 μM). PCB138 and PCB15 showed EC_{50} values of 1.48 μM and 7.51 μM, respectively. The other NDL-PCBs tested (PCB3, 8, 52, 101, and 180) presented EC_{50} values ranging from 10 μM to 100 μM. DL-PCBs were not the most toxic PCBs. PCB118 showed an EC_{50} value of 6.08 μM and PCB77 had an EC_{50} value of 31.01 μM. In the range of the concentrations tested, we have not obtained the EC_{50} values for PCBs 1, 80, 136 or 153.

For the abalone haemocyte model, the PCB that presented the most toxic value was PCB153 (EC_{50} = 1.67 μM). The other NDL-PCBs tested had EC_{50} values higher than 10 μM. For example, EC_{50} values were 21.17 μM and 89 μM for PCB107 and PCB136, respectively. For PCB3, the EC_{50} value could be not obtained because no mortality was recorded at the highest experimental con-

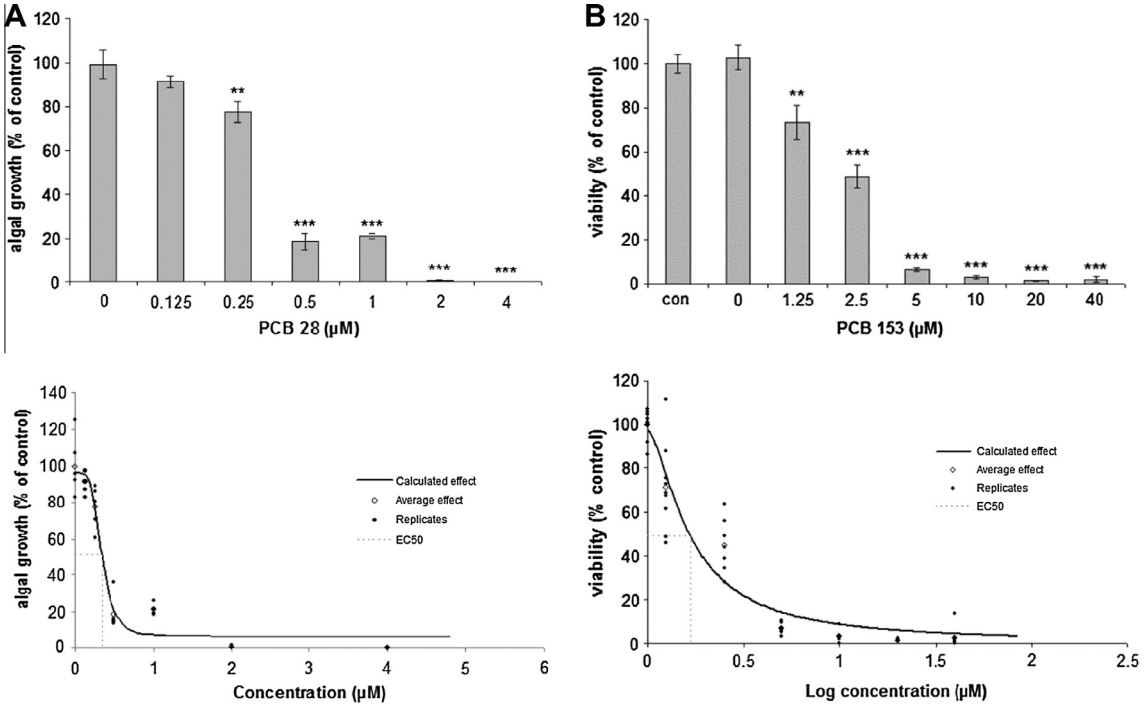

Fig. 1. Typical results of growth inhibition on algae and cytotoxicity test on abalone haemocytes. (A) Concentration-dependent effect of PCB28 on algae growth (top). Algae were exposed to PCB28 for 72 h. (B) Concentration-dependent effect of PCB153 on cell viability as determined by the MTT reduction assay (top). Each data represents the mean percentage ± standard deviation of triplicate experiments. Significant differences from control cells at $^{**}p < 0.01$ and $^{***}p < 0.001$ are given (Kruskal–Wallis tests). Calculation of EC_{50} values were performed using the Excel® macro REGTOX (bottom A and B). 0: DMSO control.

Table 2
Summary of PCB cytotoxicity (EC_{50} values) using the Excel® macro REGTOX. (A) On algae growth, (B) on abalone haemocyte viability. NA: Not Applicable. Values were expressed as μM and mg L^{-1}. The confidence intervals (95%) for each value were reported.

	EC_{50} (μM)	Confidence interval (95%)	EC_{50} (mg L^{-1})	Confidence interval (95%)
A-Results on P. subcapitata				
NDL-PCB				
PCB 1	>100	NA	>25.16	NA
PCB 3	47.37	42.61–53.91	8.91	8.01–10.14
PCB 8	15.63	15.06–17.22	3.47	3.34–3.82
PCB 15	7.51	7.50–7.52	1.67	1.60–1.62
PCB 28	0.34	0.30–0.38	0.09	0.08–0.10
PCB 52	10.05	8.24–12.34	2.91	2.39–3.58
PCB 80	>100	NA	>28.99	NA
PCB 101	19.28	12.35–66.14	6.24	4.00–21.42
PCB 136	>100	NA	>35.78	NA
PCB 138	1.48	1.26–1.79	0.53	0.45–0.64
PCB 153	>100	NA	>35.78	NA
PCB 180	47.93	32.37–76.71	18.78	12.68–30.06
DL-PCB				
PCB 77	31.01	8.29–358.17	8.99	2.40–103.83
PCB 118	6.08	2.08–12.27	1.97	0.67–3.97
B-results on H. tuberculata				
NDL-PCB				
PCB 3	>40	NA	>7.52	NA
PCB 28	89.41	63.62–143.42	22.88	16.28–36.71
PCB 107	21.17	18.78–23.31	6.85	6.08–7.55
PCB 136	20.81	17.57–24.37	7.44	6.28–8.71
PCB 153	1.67	1.48–1.89	0.59	0.53–0.67
DL-PCB				
PCB 77	47.67	24.47–187.07	13.82	7.09–54.23
PCB 169	4.31	3.52–5.59	1.54	1.26–2.00

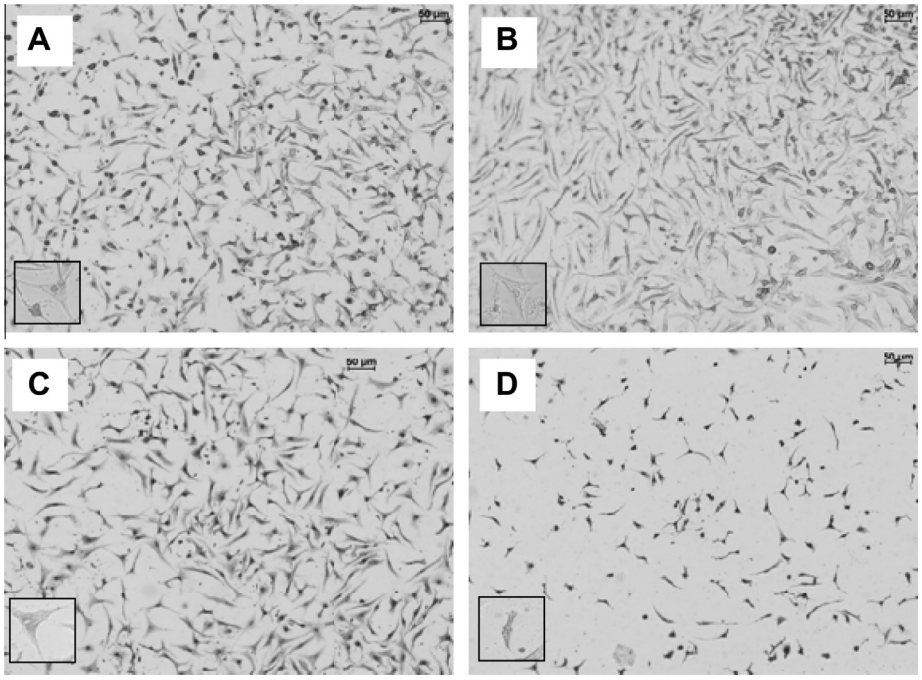

Fig. 2. Effect of PCB exposure on abalone haemocyte morphology after 10 d of culture. Cells were grown on coverslips at 17 °C. (A) control, (B) DMSO control, (C) PCB28 (1 μM), (D) PCB153 (5 μM). Bottom panel: magnification of typical haemocytes.

centration tested. For the algae experiments, DL-PCBs were not the most toxic compounds tested: PCB169 had an EC_{50} value of 4.31 μM and PCB77 had an EC_{50} value of 47.67 μM.

3.4. Structure activity relationship analysis of the result

Considering the lipophilic characteristics of PCBs, we analysed the relationship between the experimental values of toxicity and the log*Kow*, descriptor commonly used in the predictive ecotoxicity models (Fig. 3). Considering algae, EC_{50} values were significantly correlated to log*Kow* coefficients ($R = 0.71$, $p < 0.05$). This correlation appears to follow a parabolic model with increased toxicity as a function of log*Kow* for the lower chlorinated PCBs (mono, di and trichlorinated); this toxicity is reduced when log*Kow* is higher than 5.8 (Fig. 3A). The relationship between the toxicity of the PCBs on abalone and the log*Kow* values shows different

pattern: the toxic effects of PCBs which increase with log*Kow* higher than 5.7 (Fig. 3B) were significantly correlated ($R = 0.8388$, $p < 0.05$).

4. Discussion

The objective of the present study was to generate new ecotoxicological data by analysing the *in vitro* effects of PCBs based on their structural properties on two aquatic models, the green algae *P. subcapitata* and the haemocytes from the marine gastropod *H. tuberculata*. To achieve this aim, we evaluated the toxicities of 14 PCBs on *P. subca*pitata and 7 PCBs on the abalone haemocytes and compared the obtained results. The PCBs used for this study belong to both DL-PCBs (with different characteristics of planarity) and non-DL PCB.

The EC_{50} values calculated from algae data ranged from 0.34 μM for PCB28 to more than 100 μM for PCB1, 80, 136 and 153. Because most studies on the impact of PCBs on phytoplankton have focused on the bioaccumulation capacity of these compounds (Swackhamer and Skoglund, 1993; Stange and Swackhamer, 1994) and/or the effects of mixtures such as Aroclors (Conner and Mahanty, 1979; Micheli et al., 1995), there is very little data on the impact of individual PCBs on *P. subcapitata*. The work of Mayer et al. (1998) reported lower EC_{50} value than the current study: 0.2 μM and 0.015 μM for PCB31 and 105, respectively after 24 h exposure. The EC_{50} value of PCB48 was 0.134 μM after 70 h of exposure. Aroclor 1242 appeared to temporarily inhibit the growth rate of a marine diatom *Chlamydomas* (Conner and Mahanty, 1979). The severity of the response was concentration related and an inhibition of more 50% of the population was observed for concentrations ranging from 7.6 μM to 834 μM (Morgan, 1972). Thus, our results appear to be in agreement with this previous report even if the data are difficult to compare because of the methodology used (exposure, solvent, supplementation of carbon dioxide). In the range of the concentrations tested, we have not obtained the EC_{50} values for PCBs 1, 80, 136 or 153. Testing at higher concentrations is not possible due to the solubility limit of these compounds.

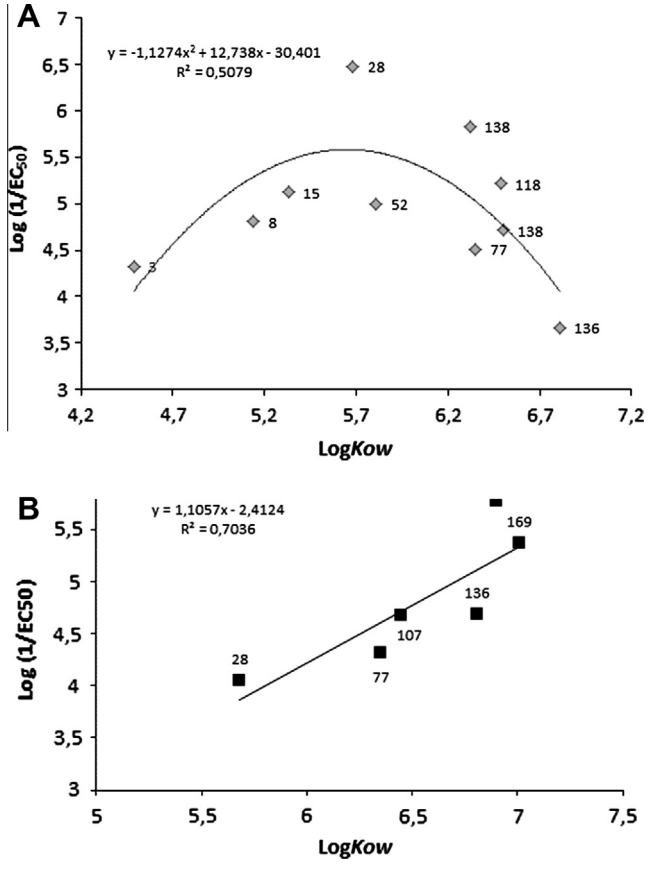

Fig. 3. Log(1/EC_{50}) experimental (μM) values as a function of PCB log*Kow*. (A) Algae values. (B) Abalone haemocyte values. Each point is tagged with the corresponding PCB name.

Considering the viability of haemocytes from the European abalone after 10 d of exposure, the results showed a toxic effect of 6 compounds (PCBs 28, 77, 107, 136, 153, and 169). The EC_{50} values calculated ranged from 1.67 μM for PCB153 to 89 μM for PCB28. EC_{50} values could not be calculated for PCB3 in the range of concentrations tested. The effects of PCBs on viability of numerous cell types of vertebrates using *in vitro* culture have already been reported in the literature. For example, Sanchez-Alonso et al. (2003) showed that the viability of rat neuronal cells exposed to 100 μM of PCB77 or 153 was decreased by 40–45% and 25–30%, respectively, after 5 h of exposure. Using mouse splenocytes, Yoo et al. (1997) demonstrated that Aroclor 1254 reduced the viability of these cells after 24 h exposure to 60%, 81% and 88% at 50, 100 and 200 μM PCBs, respectively. Thus, our results appear to be in agreement with the literature concerning the toxicities of PCB compounds. Concerning studies on molluscs, few studies have been performed to investigate the effect of PCBs on the viability of haemocyte using an *in vitro* approach. Gagnaire et al. (2006) exposed haemocytes from the oyster *Crassostrea gigas* to a range of concentrations of PCB77 from 600 nM to 60 μM and a range of concentrations of PCB153 from 70 nM to 70 μM. During this study, these authors observed no variation of mortality compared to the control which was different from the results presented in the current study. However, Gagnaire et al. (2006) exposed cells to PCBs for only a few hours (from 4 h to 24 h) compared to the 10-d exposure time of the present study. The longer exposure time allowed the cells to sensitise to the contaminant, which could explain the toxicity of PCBs on abalone haemocytes. PCBs were not the only contaminant to induce a decrease of viability on *H. tuberculata* after 10 d of exposure. Latire et al. (2012) demonstrated that the lowest concentration of cadmium that decreased the viability of such cells was 500 μg L^{-1} and the LC_{50} value of cadmium was 4100 μg L^{-1}.

Our results did not show significant differences between the DL- and NDL-PCBs toxicities whatever the studied model, contrary to what is generally observed in vertebrates. For *P. subcapitata*, PCBs 77 and 118 (DL-PCBs) have EC_{50} values in the range from 6 to 31 μM as for PCBs 3, 8, 15, 52, and 101 (NDL-PCBs). For haemocyte from *H. tubercualta*, PCBs 77 and 169 (DL-PCB) have EC_{50} values ranging from 4 to 47 μM (1–13 mg L^{-1}) as for PCBs 107, 136, and 153 (NDL-PCB). Classically, the most toxicologically active PCB congeners are those having chlorine substitution at the *para* (4 and 4′) and at least two *meta* (3,3′,5,5′) positions on the biphenyl rings but no *ortho* (2,2′,6,6′) substitution (Safe et al., 1985). The PCB congeners that have no *ortho* substitutions can assume a coplanar configuration (PCB21, 77, 126, 169). The effectiveness of specific PCB congeners as induced of different types of cytochrome-P450 dependant MFO system is determinate by their stereochemistry. Our results did not show such differences between different congeners in terms of toxicities, contrary to what is generally observed in vertebrates whatever the model used. In terms of structure, the presence of two chlorines in the *para* position does not seem to be a factor in determining the toxicities of PCBs in *P. subcapitata* or abalone haemocytes.

In the immune defense processes, the spreading of pseudopods by haemocytes and their capacity to adhere play a key role in the cell migration and the removal of foreign particles in molluscs. In the present study, some PCBs could modify the morphology of haemocytes resulting in a decrease of cytoplasmic extensions and an

increase of rounded cells. These results are in agreement with the literature dealing with the effect of contaminants on the morphology of molluscan haemocytes (Olabarrieta et al., 2001; Gomez-Mandikute and Cajaraville, 2003; Mottin et al., 2010). Such morphological changes were commonly associated with an impairment of the cytoskeleton and an intracellular disorganisation of the microfilaments as shown by fluorescent microscopy (Fagotti et al., 1996; Olabarrieta et al., 2001; Gomez-Mandikute and Cajaraville, 2003) or proteomic analyses (Chora et al., 2009). In addition, Lasserre et al. (2009) demonstrated that PCB153 affected structural proteins such as actin and tropomyosin, which may explain the morphological changes observed under microscope of MCF-7 human cells. Further studies should be performed to confirm this hypothesis in *H. tuberculata* haemocytes exposed to PCBs.

The second objective of this study was to give an interpretation in terms of the mode of action of the PCBs in the two biological models investigated in this study. Considering the lipophilic characteristics of PCBs, we observed that their toxicity is correlated with log*Kow* according to a parabolic model for *P. subcapitata*. For the less chlorinated PCBs, the toxicity increased with log*Kow* up to 5.7. From a log*Kow* of 5.7 and for most chlorinated PCBs, toxicity decreased when the log*Kow* increased. This type of pattern could be due to the structural characteristics of PCBs which could participate in the establishment of the lipid balance and the fixing of the PCBs to the membranes leading to the phenomenon of narcosis. Shaw and Connel (1984) developed a relationship between the bioconcentration of PCBs in aquatic organisms and an empirically developed steric-effect coefficient (SEC). Three structural features of the PCB molecule are of importance for the SEC: (a) the presence of 3 or 4 chlorine atoms in the *ortho* position, which twists the molecule away from planarity, (b) the presence of 3 or 4 chlorine atoms adjacent to a phenyl ring, and (c) chlorine atoms in the 3rd and 5th positions on the phenyl ring. By combining the 2 parameters *Kow* and SEC, the authors found a good measurement of the potential of different PCBs to bioaccumulate in the mullets and polychaetes. Moreover, Mayer et al. (1998) suggested that the differences in phytotoxicity of the PCBs were most likely due to the differences in the bioconcentration behaviour rather than to different toxicities. This might indicate that the phytotoxicity is due to a relatively non-specific mode of action, such as narcosis. This hypothesis was in good agreement with the pattern of the bioconcentration of PCBs by algae observed by Stange and Swackhamer (1994) suggesting that steric characteristics could be responsible for the toxicity of PCBs on account of differences in their partitioning to membrane phospholipids on *Pseudokirchneriella subcapitata*. To date, no PCB receptor has been evidenced on this green algae suggesting a no specific mode of action. Moreover, we can not exclude that the decrease of toxicity observed in our study could be due to a slower uptake. Further investigations have to be performed to determine the toxicity of PCBs with log*Kow* higher than 5.7.

Regarding abalone results, the relationship between the toxicity of PCBs and their log*Kow* showed a different pattern than what we reported for the green algae. Our experimental values demonstrated that the toxicities of PCBs increased with the octanol–water partition coefficients for log*Kow* ranging from 5.5 to 7. These results were opposite to the *P. subcapitata* results (for the same range of log*Kow* values) suggesting a different, most likely more specific mode of action than narcosis in this species. In vertebrates, it was demonstrated that biological activities of PCBs involved dependent and/or independent AhR mediated mechanisms (Fisher et al., 1998; ATSDR, 2000; Henry and DeVito, 2003). The presence of AhR in invertebrates has been shown in several phyla (Hahn, 2002) including molluscs (Butler et al., 2001; Wiesner et al., 2001; Liu et al., 2010). Recently, studies have shown that the AhR mRNA level could be used as an indicator of the exposure of bivalves to xenobiotics (Liu et al., 2010; Parisseau et al., 2011). For example, a significantly increased level of the AhR transcript was observed in haemocytes from clams 3 d after exposure to a mixture of chlorothalonil, mancozeb and benzo(a)pyrene suggesting the involvement of these xenobiotics in the haemocyte molecular pathways through AhR (Parisseau et al., 2011). On the contrary, Canesi et al. (2003) clearly demonstrated that PCBs could affect tyrosine kinase-mediated cell signalling in haemocytes from *Mytilus galloprovincialis*. More precisely, these authors showed that PCBs could impair the phosphorylation state of mitogen activated protein kinases (MAPKs) and stress activated p38. These results suggested that PCBs could also act through independent AhR mediated mechanisms in mollusc haemocytes. Further investigations have to be performed to identify the specific mode of action of PCBs (e.g., dependent or independent AhR mediated mechanisms) that we reported in this study.

Comparisons between toxicity of PCBs in abalone and algae showed similar results for PCB77 and 101. However, two PCBs, PCB28 and 153 presented opposite toxic effects. PCB28 was the most toxic compound by the *P. subcapitata* growth inhibition test, whereas PCB153 was the least toxic. On the contrary, PCB153 presented the most toxic effects by the abalone haemocyte viability test whereas PCB28 was the least toxic. Studies have shown that the sensitivity of cells to PCBs depends not only on the PCB structure but also on the cell type analysed. In that regard, Costa et al. (2007) showed that NIH 3T3 mouse fibroblasts and LNCaP human prostate carcinoma cells were more resistant to PCB126 toxicity than neuronal cells. For example, using the MTT assay as a viability test, these authors reported that the LC_{50} values for PCB126 in 3T 3 and LNCaP cells were higher than 100 μM after a 24 h exposure whereas in Purkinje and hippocampus cells, the LC_{50} value for PCB126 were 2.2 μM and 19.9 μM, respectively. Further investigations should be performed to determine the differences of toxicity we observed in the two models used in this study.

There is limited information on bioaccumulation and toxicity of PCBs for algae or abalone and evidence on population and community-level effects is rare (Sibley and Hanson, 2011). Paterson et al. (2003) evaluated the historical response of diatoms and chlorophytes communities in sediment cores from a PCB-contaminated freshwater lake. Despite peak of contamination little change in either community was observed through time. They hypothesised that the bioavailable fraction of PCBs in lake sediments was too low to cause detrimental effects in the limnetic phytoplankton communities. The PCB concentration in the water column ranged from 3.45 and 56 ng L^{-1} in the Newark bay estuary, USA (Dimou et al., 2006) and from 0.02 to 14.8 ng L^{-1} in the Pearl river estuary, China (Chen et al., 2011). In Europe, PCB contamination of the water column ranged from 25 to 64 ng L^{-1} and from 23 to 108 ng L^{-1}, respectively, in the surface and bottom waters in the Seine estuary, France (Cailleaud et al., 2009). In our study, the PCB concentrations that induced significant toxic effects ranged from 0.1 to higher than 35 mg L^{-1} which represent several orders of magnitude higher than actual environmental concentrations, regardless of the model used. These data indicate that the impact of PCBs on plants and algae are low due to current environmental concentrations, but they can serve as important sources of PCBs for organisms at higher trophic levels.

5. Conclusions

In summary, the present study demonstrated that PCBs affect the green alga, *P. subcapitata* and the haemocytes from the European abalone, *H. tuberculata*. Our results report new ecotoxicological data using a conventional and non-conventional biological model. Moreover, we did not show differences between the

toxicity of the tested DL- and NDL-PCBs suggesting that the presence of two chlorines in the *para* position of PCBs is not as important with regards to toxicity in our invertebrate models as in vertebrate models. Nevertheless, the PCB concentrations that induced significant toxic effects were at several orders of magnitude higher than actual environmental concentrations ($\sim$10 ng L^{-1}), regardless of the model used. Our toxicity-log*Kow* relationship investigations highlighted that PCBs impaired algal growth *via* a non-specific mode of action (e.g., narcosis), whereas, in haemocytes, a different most likely more specific mode of action (e.g., dependent or independent AhR mechanism) was suggested. These differences in the mode of action of PCBs highlight the importance of using marine invertebrate models to assess the potential toxicity of compounds found in the marine environment.

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